Results: BAP level was higher in OA synovial fluid than in simple synovitis, and d-ROM level was slightness lower in OA than control synovitis, but this differences were not significant. BAP and d-ROM levels were not related with OA grade.
After intra-articular injection of HA, BAP level was significantly increased and d-ROM level was reduced. On the other hand, BAP and d-ROM level were significantly decreased after injection of corticosteroid.
Conclusions: Oxidative stress and anti-oxidative potential in synovial fluid were not significant difference between OA and simple synovitis. Our results showed that hyaluronan increase the anti-oxidant potential and decreased oxidative stress, but corticosteroid decreased both oxidative stress and the anti-oxidative stress potential.

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INHIBITION OF PROSTAGLANDIN E2 BY CELECOXIB DECREASES GLYCOSAMINOGLYCAN RELEASE, HOWEVER DOES NOT STIMULATE REPAIR OF OSTEOARTHRITIC CARTILAGE TISSUE

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Purpose: The pro-inflammatory lipid mediator prostaglandin E2 (PGE2) is present in the osteoarthritic joint and is know to play a role in osteoarthritis (OA). Both stimulated chondrocytes and synovial fibroblasts are capable of producing PGE2, however, to which extent osteoarthritic joint tissue is involved in its production and if the PGE2 produced has an effect on cartilage degeneration and regeneration is not entirely clear. Recently, we set up a coculture model with OA cartilage and OA synovial tissue that demonstrated to be more representative for OA than cartilage explant monocultures. To further clarify the role of PGE2 in osteoarthritis, the effect of blocking COX-2 and hence production of PGE2, on cartilage degeneration and cartilage regeneration was studied in the coculture model and in a chondrocyte 3D regeneration model, respectively.
Methods: OA cartilage and OA synovial tissue were cultured alone or in coculture for 21 days with the addition of celecoxib at 0.1, 1 and 10 μM. Cartilage regeneration in the presence of absence of 1 μM celecoxib, was studied by seeding healthy or osteoarthritic chondrocytes (P2) at high density on collagen-coated filters and cultured for 28 days. As outcome parameters, glycosaminoglycan (GAG) release and content, safranin-O staining and PGE2 production were determined.
For statistical analysis, univariate analysis of variance was performed. To correct for inter-donor variability a randomized block design was used. P values <0.05 were considered significant.
Results: Both osteoarthritic cartilage and synovial tissue produced PGE2, albeit at much higher amounts by synovial tissue. Celecoxib inhibited PGE2 production (Fig. 1) and at 10 μM decreased glycosaminoglycan (GAG) release in coculture (Fig. 1), however, had no effect on GAG content in either cartilage monoculture or coculture with OA synovial tissue (Fig. 1).
During regeneration, OA chondrocytes produced low amounts of PGE2, which also could be inhibited by celecoxib (Fig 2; $P < 0.05$). However, celecoxib had no effect on cartilage regeneration in either the healthy and osteoarthritic chondrocytes (Fig. 2).
Conclusions: Osteoarthritic cartilage explants, OA chondrocytes and in particular OA synovial tissue produced PGE2, which could be inhibited by celecoxib, suggesting COX-2 was the main COX active in the tissue. In coculture, celecoxib inhibited both PGE2 production and GAG release, although no effect on GAG content was observed. During cartilage regeneration, the decrease of the PGE2 production was not accompanied by an effect on chondrocyte metabolism. This study shows that celecoxib inhibits PGE2 production in both explant and regeneration culture, however, its effect on cartilage metabolism is limited.

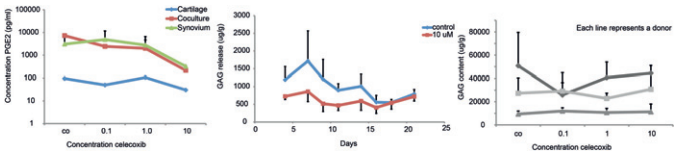

Fig. 1. PGE2 production, GAG release and GAG content in a coculture model with OA cartilage and synovial tissue.

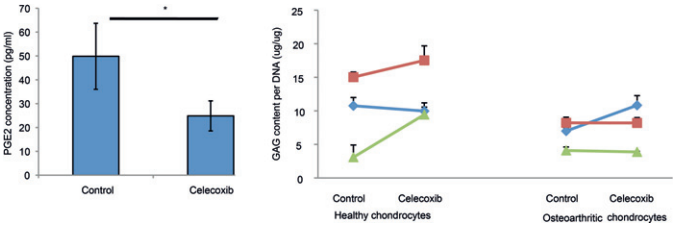

Fig. 2. Celecoxib decreases PGE2 production in OA chondrocytes (*$P < 0.05$), however without effect on GAG content.

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MILD ELECTRICAL STIMULATION WITH HEAT SHOCK INCREASES HEAT SHOCK PROTEIN 70 AND PROTEOGLYCAN CORE PROTEIN IN ARTICULAR CARTILAGE

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Purpose: Heat shock protein 70 (HSP70) inhibits the apoptosis of chondrocytes and has the protective effect on the cartilage. Meanwhile, we reported that mild electrical stimulation (MES) increased HSP70 by attenuating proteasomal degradation and that heat shock (HS) accelerated the translation of HSP70 in articular chondrocytes. The purpose of this study is to investigate the effect of MES in combination with heat shock (HS) on HSP70 in articular cartilage.
Methods: Sprague-Dawley rats (8 weeks of age, 200 g) were divided into 4 groups: Control group, MES group, HS group and HS+MES group. Rats were anesthetized and electrical stimulation was delivered to their left knee through a pair of 2.5 cm diameter electro-conductive and thermo-generative rubber electrodes (45°C) for 30 minutes. The electrodes were connected to a Biometronome (Tsuchiya Gum Co., Ltd., Kumamoto, Japan) that delivered 12 V (55 pps) of direct current with individual pulse duration of 0.1 ms. For the control group, rats were sham treated as above for 30 minutes per session but without conducting HS+MES. Eight hours after the treatment, protein lysates were obtained from cartilage fragments and subjected to Western blotting for HSP70 and ubiquitinated proteins ($n = 4$ each). Total RNA was isolated from the cartilage 24 hours after the HS+MES treatment. Relative expressions of mRNA for HSP70 and proteoglycan core protein (PG) were examined by real-time PCR ($n = 6$ each).
Results: After the treatment, we cannot find any adverse effect such as burn injury or gait disorder. Western blotting showed that HS modestly augmented HSP70. We found a remarkable increase of HSP70 and ubiquitinated proteins in HS+MES treated rats compared with the other groups (Figure 1). Real-time PCR analysis proved that MES did not affect the HSP70 mRNA. Compared to the control group, the HSP70 mRNA expression increased in the HS group and HS+MES group, and there was no significant difference between these two groups (Figure 2A). The expression of PG mRNA was significantly enhanced in the HS+MES group (Figure 2B).
Conclusions: In this study, HSP70 and PG mRNA in articular cartilage significantly increased by MES in combination with HS. HSPs constitute a family of highly conserved proteins which are synthesized in cells after stress loading including heat stress. HSP70 not only protect cells from various forms of stress, but also facilitate the recovery from stress-induced cell injury as molecular chaperones. We have reported that adenoviral overexpression of HSP70 resulted in promotion of PG transcription in chondrocytes, protection of chondrocytes from heat stress, and inhibition of NO-induced apoptosis of chondrocytes. Moreover, plasmid delivery of HSP70 in rat patellar cartilage decreased the severity of osteoarthritis-lesions. In the HS+MES group, PG mRNA could be induced by the HSP70. From the clinical point of view, effective and less-invasive method of HSP70 induction in articular chondrocyte could contribute to the treatment of osteoarthritis. MES and HS have already used as the safety method of HSP70 induction in vivo and attenuates hepatic ischemia/reperfusion injury. It is reported that MES induce HSP70 and ubiquitinated proteins via attenuation of proteasomal degradation. The elevation of HSP70 and ubiquitinated proteins in articular cartilage could be also due to the attenuation of proteasomal